

IMPACT OF CADMIUM AND ZINC ON ROOT SYSTEM OF LOLIUM PERENNE AND TRIFOLIUM REPENS

T. LAMBRECHTS, G. LEQUEUE, G. LOBET, S. LUTTS

Earth and Life Institute, Université catholique de Louvain, Croix du Sud 4-5 (L7.07.13), 1348,
Louvain-la-Neuve, Belgium

INTRODUCTION

Urbanization, industrialization and agricultural practices have resulted in soil contamination with heavy metals in many world areas. Phytostabilization is an emerging solution to limit pollutant dispersion out of the contaminated area without expensive costs (Vangronsveld et al., 2009). However, because of the heavy metal phytotoxicity, the implementation of a plant cover could be jeopardized. Indeed, many studies have demonstrated the negative impact of heavy metals on shoot growth and development. Impact on roots is less understood, because of the difficulty to access to this plant compartment. However, setting up of an important root system is crucial for phytostabilization strategy, in order to limit metal bioavailability, metal leaching and soil dispersion (Vangronsveld et al., 2009), but also to guarantee the durability of the plant cover.

In this study, we focused on cadmium and zinc, two common metal pollutants in Belgian industrial soils. Two plant species, *Lolium perenne* and *Trifolium repens*, exhibiting drastically different root systems, were selected to assess the mid-term impact of the two heavy metals on roots, thanks to analyses of root system dimensions (weight, length, diameter) and physiological measurements.

MATERIAL AND METHODS

Two different experiments were performed into phytotron under fully controlled environmental conditions (16h photoperiod, 24°C day, 22°C night, relative moisture 80%, light intensity 100µmol/m²/s). Two weeks after sowing in loam pot, plant seedlings were transferred into two kinds of tube pot (1 plant per pot) filled with washed quartz and were allowed to grow during 4 weeks. The first pots were columns of 55cm height and 25cm diameter, designed to study root system with depth and previously tested to not modify root architecture ; the second ones were smaller (13cm diameter, 16cm height) and were used to perform some physiological measurements. Every day, pots were irrigated with Yoshida nutrient solution, contaminated or not with heavy metals. Solution volumes were adapted for pots and columns, to get the same volume ratio solution/pot. The following metal concentrations were selected based on preliminary experiments: for *Lolium perenne*, Cd 25 and 50µM, Zn 500 and 1000µM; for *Trifolium repens*, Cd 5 and 10 µM, Zn 50 and 100µM. Each week, pots were flushed with deionized water to avoid metal accumulation in the substrate. All the treatments were performed with replications for small pots (n=5) and for columns (n=3).

Small pots were used for shoot and root fresh and dry weight determination, as well as for measurement of metal concentration. Columns were harvested according to depth: successive substrate slices of 5cm were harvested and roots were separated from quartz, fresh weighted and stocked in FAA solution. Roots were then scanned and the obtained images analysed with open source software ImageJ. A macro was built with algorithms and logical operations to routinely get root length density and root diameter.

Another experiment was next performed to go deeper into the analysis of metal impact on roots. All the experimental conditions were the same as for

the “small pot” experiment. After 4 weeks of treatment, roots of half of the pots were harvested, oven dried then crushed with mortar and liquid nitrogen to get fine powder. Concentrations of lignin and structural polysaccharides (cellulose and hemicellulose) were assessed through Van Soest method (Van Soest et al., 1991). The other root systems were stocked into FAA solution for further histochemical analyses. Cross-sections were performed on random roots at 1cm below the crown and stained with safranin-fastgreen (lignin, suberin and cutin in red, cell walls and cytoplasm in blue-green). Analysis of root diameter and lignification percentage was performed thanks to image analysis software ImageJ. Finally, specific measurements of cell viability were performed on freshly harvested *Lolium perenne* roots, through the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) into formazan (Lutts et al., 2004). Results of this measurement were compared to another technic to assess cell viability by propidium iodide staining. This chemical compound is able to penetrate damaged cell membranes and to intercalate with DNA and RNA to form a bright-red fluorescent complex seen in dead cells (Lequeux et al., 2010).

RESULTS

Results of this extended abstract focuses on *Lolium perenne* only. Heavy metals clearly decreased root length density at all depths (Figure 1). However, Cd 25 μ M differed from the other pollutant treatments, as the decrease of length was less marked below the surface layer. Mean root diameter (Figure 2) was also affected by heavy metals, with an increase of diameter at the surface of the layer, followed by a strong decrease below. It differed drastically from the control which showed similar values of diameter along almost all the depth. As for the root length, Cd 25 μ M differs from the others contaminated treatments, with an increase of root diameter for the first 30cm of substrate depth.

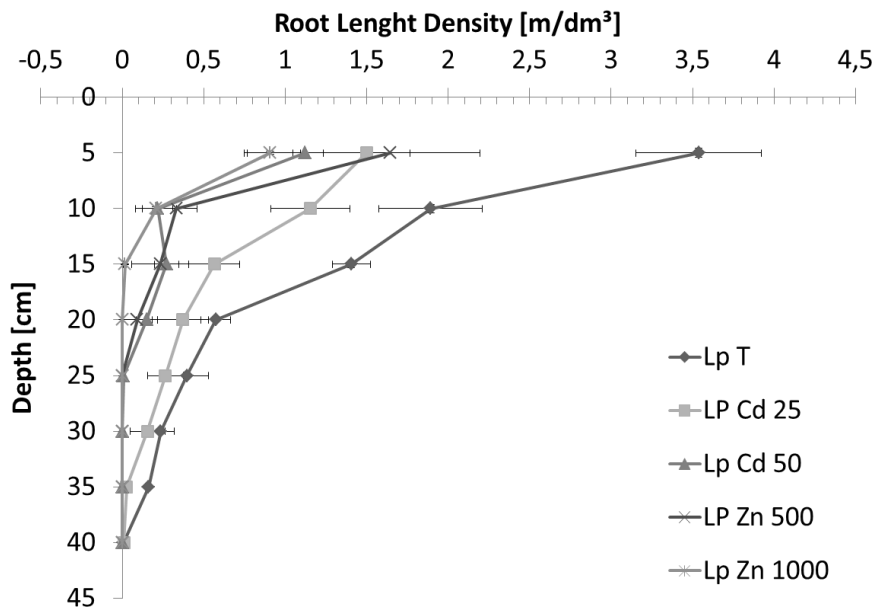


Figure 1. Root length density [m/dm³] vs. depth [cm] for *Lolium perenne* after 28 days of exposure to different nutrient solutions: LP T (control without metal contamination), Cd 25 μ M, Cd 50 μ M, Zn 500 μ M, Zn 1000 μ M

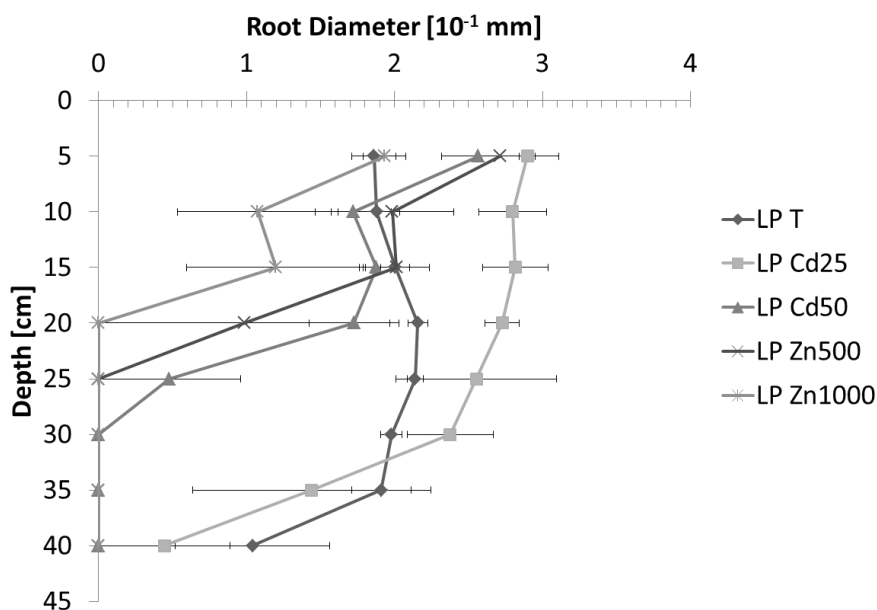


Figure 2. Root diameter [10-1mm] vs. depth [cm]

These results can be linked to metal concentrations in shoots and roots of ryegrass (Table 1). Roots accumulated at least ten times more Cd than shoots, and 5 times more Zn for polluted treatments. Increase of metal concentration in irrigation led to significant higher Cd concentration in roots, but not for Zn.

Table 1. Cadmium (Cd) and Zinc (Zn) concentrations [mg/kg DW] in shoots (S) and roots (R). Values sharing the same letter are not statistically different at $p=0.05$. BLD = below the limit of detection.

		Control	Cd25	Cd50	Zn500	Zn1000
Cd	S	BLD	86±16 b	135±24 a	BLD	BLD
	R	BLD	1279±261 b	2299±388 a	BLD	BLD
Zn	S	134±18 c	146±24 c	129±9 c	953±131 b	1403±99 a
	R	157±25 b	190±27 b	296±77 b	5258±777 a	7174±1949 a

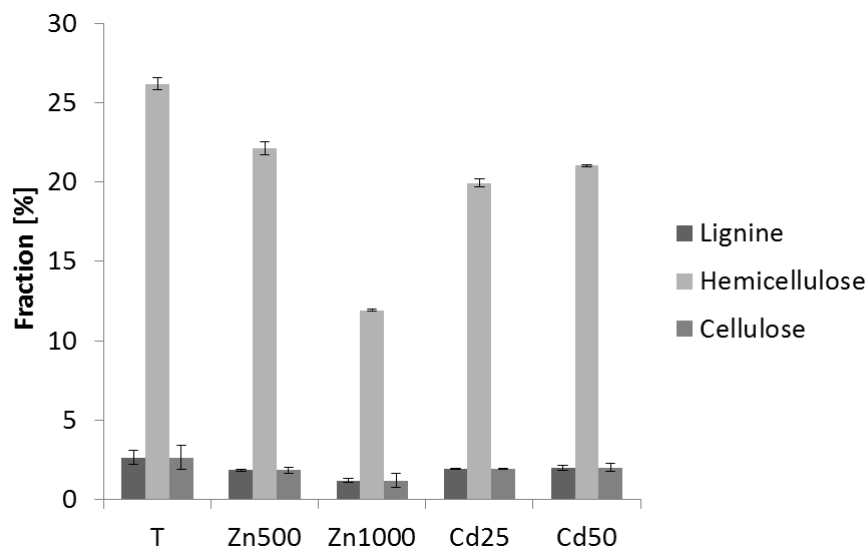


Figure 3. Fraction [%] of lignin, hemicellulose and cellulose detected with Van Soest method in roots

Metal contamination induced a decrease in root structural polysaccharides and lignin content (Figure 3). This modification was evident for Zn 1000 μ M. Histochemical analyses (data not shown) however indicated for Cd treatments an increase in root diameter just below the plant crown, correlated with an increase in lignification percentage. Heterogeneity of the adventitious roots population was especially high for Zn-treated plants, and the detected increase of root diameter could not be linked to lignin percentage in this case.

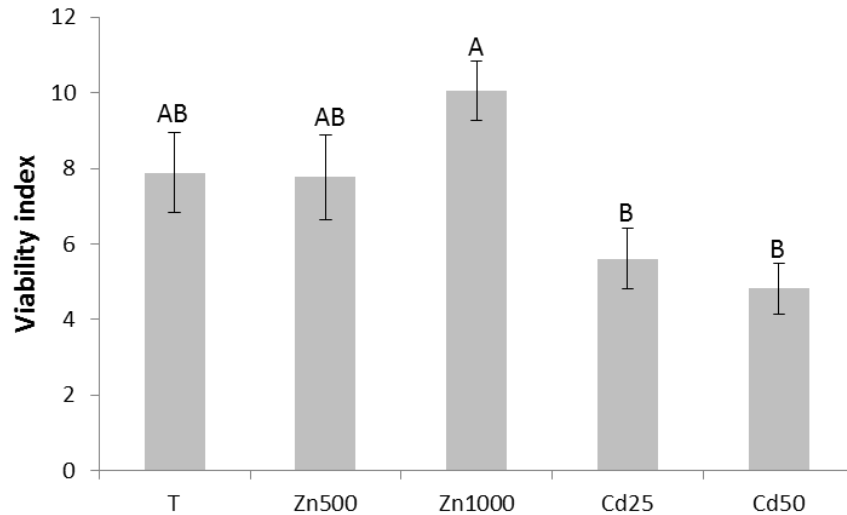


Figure 4. Index of viability for freshly harvested apex roots

Cadmium decreased the cell viability in the apex of contaminated roots (Figure 4), but zinc had the opposite effect, especially for Zn 1000 μ M. These results were confirmed by propidium iodide staining (results not shown), which revealed a higher number of red fluorescent apex roots for cadmium treatments comparatively to control, and an opposite effect for zinc.

DISCUSSION

Several studies showed that *Lolium perenne* is an adequate plant species for phytostabilization, since it tolerates high levels of metal soil contamination while limiting metal translocation to shoots (Bidar et al., 2009). However, metals accumulated in roots had strong impacts on root system. They indeed decreased the total root biomass by limiting the root depth and reducing root length. This impact could limit the plant efficiency in phytostabilization strategy.

Both Cd and Zn increased mean root diameter in the top soil, in relation to the presence of a high number of short and wide adventitious roots. Nevertheless, Cd and Zn had contrasting different effects. First, cadmium induced formation of wide roots with higher lignification level than control ones. Such a lignin deposition was observed with other plant species (Ederli et al., 2004). In the same time, these roots showed higher cell mortality, in contrast to earlier report by Delp  r  e and Lutts (2008) for *Solanum lycopersicum*. However, this lignin deposition occurred locally just below the crown while the total lignin content of the whole root system decreased in comparison to control. Because of a high heterogeneity in lignin content of short adventitious roots, higher lignin deposition was not detected with zinc treatments. Higher cell viability was observed for Zn 1000 μ M than for Cd-treated roots. Such reduced primary root elongation and radial swelling without decreased cell viability was also observed with *Arabidopsis thaliana*

mutants exposed to high nitrate or chloride concentrations (Hermans et al., 2010).

CONCLUSION

A plant tolerant to heavy metal soil contamination and limiting metal translocation is not necessary well-adapted to phytostabilization, as metals could affect root architecture. If Cd and Zn decreased both root depth and length, and increased root diameter, some physiological modifications seemed to differ between the 2 metals considered.

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